A forward role for Hedgehog

Sonic hedgehog, a midline signal secreted by the notochord to control pattern in the spinal cord, is also implicated in development of the forebrain and eyes; it acts within a positional framework to produce many cell types.

The bilateral organization of the vertebrate nervous system is underlain, ontogenically as well as topologically, by the notochord. Derived from mid-dorsal mesoderm, the notochord is a compression-resisting rod that plays a supporting role as the axial skeleton in larval or embryonic vertebrates and later as a template for the basicranium and vertebrae. Earlier in development, the notochord plays what is arguably a leading role — as an organizer of nervous system development.

Control of ventral pattern in the central nervous system by the notochord

Over the past few years, a wealth of data from tissue recombination, transplantation and genetic experiments has demonstrated that the notochord is a signalling centre for patterning the neural tube (Fig. 1). Induction by the notochord results in the formation of a specialized non-neural midline zone in the overlying neurectoderm, called the floor plate, and also in the specification of motor neurons in the bilaterally adjacent neural plate [1,2]. Whereas floor plate induction seems to require intimate contact [3], motor neuron induction can be achieved at a distance [4], suggesting that a diffusible molecule is involved. At a slightly later developmental stage, the floor plate itself acquires the same inductive capabilities: it can also induce motor neurons and it will homeogenetically induce itself [2].

The ability of midline signals to influence the development of the ventral (basal) region of the neural tube is not restricted to the spinal cord: they are also influential in both the hindbrain and midbrain. Besides inducing motor neurons and floor plate at these more anterior levels of the neuraxis, midline signals are also involved in the development of region-specific neuronal subpopulations: serotonergic neurons of the hindbrain raphe nucleus [2] and dopaminergic neurons of the midbrain substantia nigra [5] both develop close to the floor plate (Fig. 2) and can be induced to form in competent neural plate by contact with notochord grafts or floor plate explants, respectively. The interpretation of the midline signal is determined by the responding tissue; thus, serotonergic neurons can be induced in ectopic regions of the anterior hindbrain by notochord from more posterior axial levels [2] and, similarly, the induction of dopaminergic neurons in midbrain neuroepithelium can be triggered by floor plate from different axial levels [5]. These studies demonstrate that the same inductive signals are produced all along the axis by the midline tissues, and that the specific outcome depends on the anteroposterior position of the responding tissue.

Sonic hedgehog — a morphogen that specifies diverse ventral cell fates

Although motor neuron and floor plate induction appear to require different signals, one diffusible and the other contact-dependent, molecular studies have shown that a single molecule can account for both processes. The gene *Sonic hedgehog* (*Shh*) not only displays the appropriate expression dynamics, being expressed first in the notochord and then the floor plate [6,7], but the amino-terminal autoproteolytic cleavage product of the SHH protein is sufficient to induce both motor neurons and floor plate cells in a concentration-dependent manner [8,9]. Evidence that SHH is required for floor plate induction comes from the use of blocking antibodies [8].

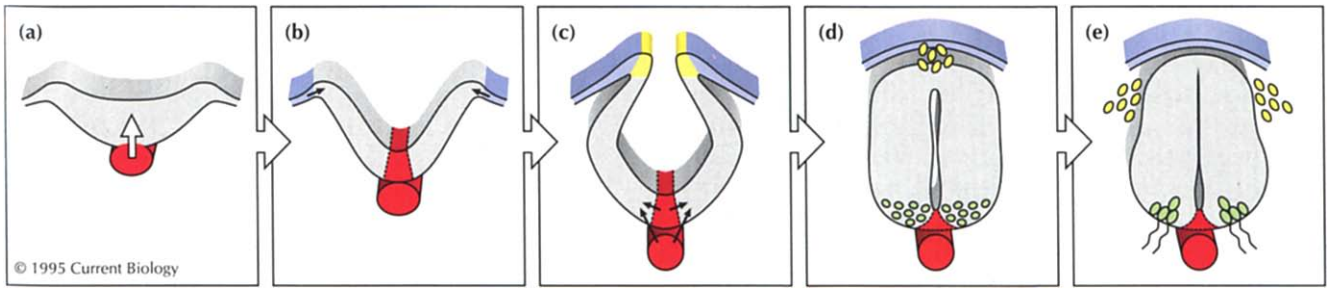

Fig. 1. Stages in the formation of spinal neural pattern, seen in transverse section. **(a)** The notochord underlies the neural plate and expresses *Shh* (red). **(b)** Notochord-derived SHH induces the differentiation of floor plate, which also expresses *Shh*. *Bmp-7* (blue) is expressed in epidermal ectoderm adjoining the neural plate. **(c)** As the neural plate closes, *Slug*, an early marker for neural crest (yellow), is expressed at the junction between neural and epidermal ectoderm. **(d)** In the early neural tube stage, *Isl-1*-expressing cells (green) appear close to the floor plate, and neural crest cells (yellow) leave the dorsal tube and midline ectoderm through breaks in the basal lamina. **(e)** Finally, motor neurons (green) differentiate in the ventral cord.

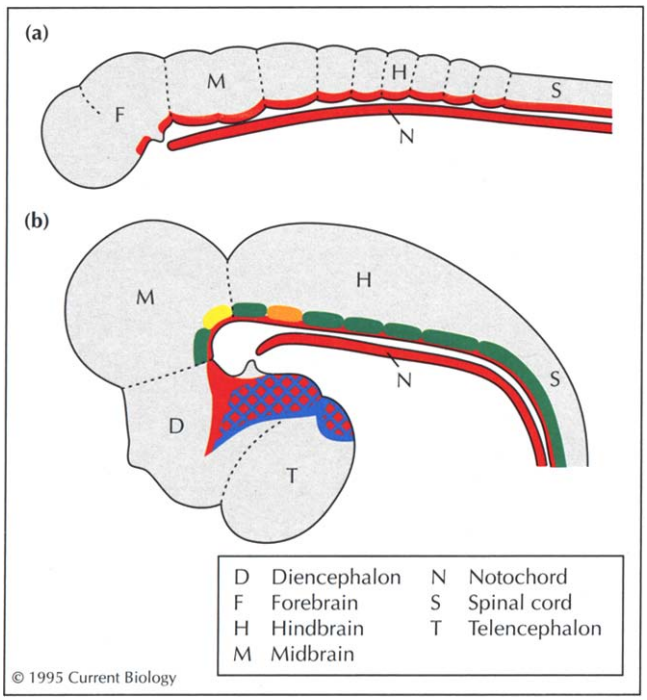

Fig. 2. Stages in the formation of anterior pattern in the central nervous system, seen in lateral view. **(a)** Soon after neural tube closure; the notochord underlies spinal cord, hindbrain, midbrain and posterior forebrain, where its tip lies close to the infundibulum. *Shh* (red) is expressed by both notochord and mid-ventral neural tube cells. **(b)** Later, *Shh* expression extends in the rostral diencephalon (D) and telencephalon (T). At midbrain and hindbrain levels, *Isl-1*-positive motor neurons (green), serotonergic neurons (orange) and dopaminergic neurons (yellow) differentiate adjacent to the *Shh*-expressing ventral midline cells. In the forebrain, the expanded domain of *Shh* expression is also associated with *Isl-1* expressing cells (blue).

As with the results obtained from notochord–floor plate co-cultures and transplantations, the response to SHH signalling depends on the anteroposterior position of origin of the responding tissue: for example, recombinant SHH induces dopaminergic neurons only in explants of midbrain neuroepithelium [10]. Furthermore, when SHH signalling is antagonized by raising the activity of the cAMP-dependent protein kinase A, induction of dopaminergic neurons by floor plate is blocked [10] — suggesting that SHH may be necessary, as well as sufficient, for inducing midbrain dopaminergic neurons.

The extent of motor neurons along the anteroposterior axis corresponds with that of their inducing tissues, and both have an anterior limit close to the midbrain–forebrain junction (Fig. 2). Thus, the forebrain (telencephalon and diencephalon) is devoid of motor neurons and, in all regions but its most posterior, it has no floor plate, nor is it underlain by notochord. The absence of these midline structures therefore raises the question of how the bilateral organization of the forebrain and the differentiation of its ventral cell types are controlled. It appears, however, that even in this terminal expansion of the central nervous system a common mechanism is used for ventral patterning. *Shh* is expressed along the ventral midline of the forebrain (extending into bilateral wings in the diencephalon; see Fig. 2) [11], and ventral forebrain cells express the gene *Isl-1*, an early marker of motor neuron specification in the more caudal regions of the central nervous system.

Expression of *Isl-1* in the forebrain suggests, however, that *Isl-1* is a general marker of ventral character rather than a specific marker of motor neurons. Forebrain explants that are cultured away from ventral midline tissue do not develop *Isl-1*-expressing cells, but they will do so when co-cultured with COS cells that have been transfected with *Shh* [11]. The *Isl-1* positive cells induced by this treatment are forebrain-specific, in that they express *Nkx-2.1* and do not express any of the markers indicative of motor neurons. Whereas *Shh* is expressed in the ventral regions of both the diencephalon and telencephalon, it appears that the diencephalic domain is influential in controlling cell patterning in the ventral forebrain as a whole: *Shh* is expressed in the ventral diencephalon considerably before the appearance of *Isl-1*-positive cells in either forebrain region, and studies *in vitro* have shown that the midline rostral diencephalic cells can induce *Isl-1* expression in telencephalic cells [11]. By contrast, the onset of telencephalic expression is later in development, apparently after the induction of *Isl-1*-positive cells in that region has occurred. It is possible that planar SHH signalling extends anteriorly from the diencephalon into the telencephalon.

Involvement of Sonic hedgehog in eye development

Genetic studies in zebrafish have also identified the ventral midline of the diencephalon as a controlling region for patterning the anterior end of the nervous system. This midline territory is deleted in the *cyclops* mutant, whose phenotype most obviously involves fusion of the eyes around the anterior pole of the embryo. Molecular analysis of the early *cyclops* phenotype has revealed that there is no *Shh* expression in ventral midline structures of these embryos [12], presenting the possibility that the normal expression of *Shh* has consequences that extend beyond patterning the ventral neural tube. In homozygous *cyclops* embryos the optic stalk — a region that normally expresses *Pax-2* — is diminished, whereas the retina, which normally expresses *Pax-6*, extends throughout the optic territory such that the eyes are fused not by optic stalk tissue but by retina [13].

That SHH has a role in patterning the eye has been demonstrated in ectopic over-expression studies by *Shh* mRNA injection into the one-cell-stage embryo [14,15]. These embryos have phenotypes that are reciprocal to those seen in *cyclops*: the domain of *Pax-2* expression is extended and encroaches into the territory that would normally be *Pax-6*-positive and, at the cellular level, the over-expressing embryos have an enlarged optic stalk and a reduced retina. Although the consequences of interfering with *Shh* signalling have yet to be fully analyzed in eye development, these studies indicate that *Shh* is responsible for patterning not only the ventral forebrain but also the optic territories.

Positional information on the anteroposterior and dorsoventral axes

How can a single molecule exert such widespread control of such diverse cell pattern? The studies we have cited are consistent in showing that *Shh* alone is sufficient to account for the inductive activities of ventral midline structures, and that the specific consequence of its action depends on the responding tissue. *Shh* must be a general signal that is interpreted in a specific way by the individual competence of an induced tissue — the patterning activities of *Shh* work within the context of previously established anteroposterior positional clues and previously specified regional identity (Fig. 3). That this is indeed the case has already been demonstrated experimentally in the hindbrain of the chick embryo. The hindbrain is subdivided along the anteroposterior axis into metameric units, rhombomeres, each of which has a unique identity. In particular, rhombomere 4 is marked by a high level of expression of the *Hoxb-1* gene [16] and, at a later stage, by the emergence of a unique cell group adjacent to the floor plate, the contralateral vestibulo-acoustic (CVA) efferent neurons [17].

When rhombomere 4 is transplanted to a more rostral position (in place of rhombomere 2), it maintains *Hoxb-1* expression and CVA neurons are produced. Furthermore, the CVA neurons are produced by dorsal rhombomere 4 tissue if it is placed close to the ventral midline in this ectopic anteroposterior location — the rhombomere 4-specific ventral cell types are formed irrespective of the dorsoventral level of origin of the graft [18]. It appears that cells are first assigned their anteroposterior identity, but that their ultimate choice of fate has to await a later midline signal. The multipotency of precursor cells is restricted first to a repertoire appropriate to their anteroposterior position, leaving them in a state of competence to respond to midline signals that only later determine specific cell identity appropriate to dorsoventral position (Fig. 3). The signals involved in establishing anteroposterior identity remain elusive, but a gradient of retinoic acid emanating from Hensen's node is one candidate [19].

Dorsalizing signals

As far as signals operating on the dorsoventral axis are concerned, the ventral midline expression of *Shh* represents only half the story, and currently progress is being made towards identifying dorsally derived signals. Recent work has demonstrated that dorsal fates are influenced by an interaction between the neurectoderm and the epidermal ectoderm [20–24], which results in the induction of neural crest cells and of dorsal neural tube markers. At a molecular level, this interaction appears to be mediated by members of the bone morphogenetic protein (BMP) family of signalling molecules, in particular BMP4 and BMP7 [24]. Genes expressing both BMPs are expressed in the dorsal epidermal ectoderm, and both recombinant proteins mimic the inducing ability.

These dorsal signals, together with a related protein, dorsalin-1 [25], which is expressed in the dorsal neural tube, may act as opponents of ventral SHH signalling; it is possible that specification of the entire range of cell fates down the dorsoventral axis of the neural tube may be determined by their antagonistic activities (Fig. 1). What remains to be shown is whether the proteins secreted from either pole form extracellular concentration gradients down the dorsoventral axis, or whether a cell–cell relay is involved. It will also be interesting to discover whether the dorsalizing action of BMPs is manifest along the anteroposterior extent of the neuraxis, and whether it is also involved in patterning the eye. If so, it might be expected that BMP activity would promote *Pax-6*-expressing cells and repress *Pax-2*-positive cells. If BMPs do represent a single dorsalizing activity, undoubtedly they will operate, like SHH, within the framework of already established anteroposterior positional cues.

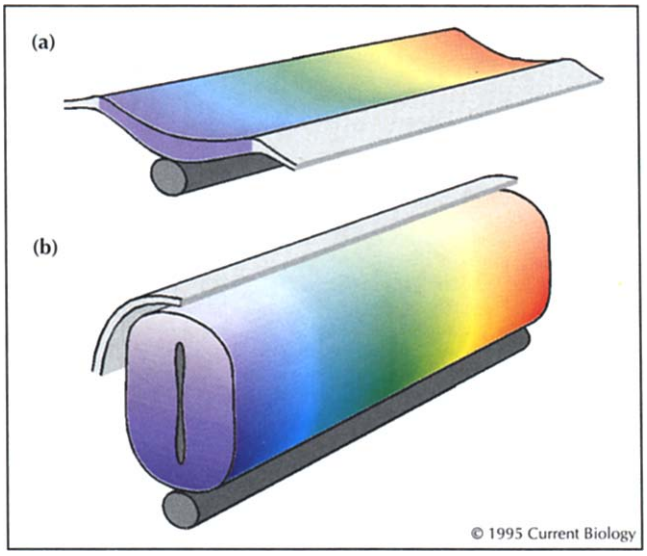

Fig. 3. A coordinate system of positional information in the neural tube. In **(a)**, positional information along the anteroposterior axis of the neural plate has already been established — different anteroposterior positional values are represented by different colours — but there is as yet no dorsoventral coordinate. This is established later, in **(b)**, where each level of the anteroposterior axis receives signals from both the ventral pole (SHH, dark grey) and the dorsal pole (BMP7, light grey) — different dorsoventral positional values are represented by different colour intensities. See [18].

References

1. van Straaten HMW, Hekking JMW, Wiertz-Hoessels EL, Thors F, Drukker J: **Effect of the notochord on the differentiation of a floor plate area in the neural tube of the chick embryo.** *Anat Embryol* 1988, **177**:317–324.
2. Yamada T, Placzek M, Tanaka H, Dodd J, Jessell TM: **Control of cell pattern in the developing nervous system: polarising activity of the floor plate and notochord.** *Cell* 1991, **64**:635–647.
3. Placzek M, Jessell TM, Dodd J: **Induction of floor plate differentiation by contact-dependent, homeogenetic signals.** *Development* 1993, **117**:205–218.
4. Yamada T, Pfaff S, Edlund T, Jessell TM: **Control of cell pattern in the neural tube: motor neuron induction by diffusible factors from notochord and floor plate.** *Cell* 1993, **73**:673–686.
5. Hynes M, Poulsen K, Tessier-Lavigne M, Rosenthal A: **Control of neuronal diversity by the floor plate: contact-mediated induction of midbrain dopaminergic neurons.** *Cell* 1995, **80**:95–101.
6. Echelard Y, Epstein DJ, St-Jacques B, Shen L, Mohler J, McMahon JA, McMahon AP: **Sonic hedgehog, a member of a family of putative**

signalling molecules, is implicated in the regulation of CNS polarity. *Cell* 1993, **75**:1417–1430.
7. Roelink H, Augsberger A, Heemskerk J, Korzh V, Norlin S, Ruiz i Altaba A, Tanabe Y, Placzek, M, Edlund T, Jessell TM: **Floor plate and motor neuron induction by *vhh-1*, a vertebrate homolog of *hedgehog* expressed by the notochord.** *Cell* 1994, **76**:761–775.
8. Marti E, Bumcrot DA, Takada R, McMahon AP: **Requirement of 19K form of Sonic hedgehog for induction of distinct ventral cell types in CNS explants.** *Nature* 1995, **375**:322–325.
9. Roelink H, Porter J, Chiang C, Tanabe Y, Chang DT, Beachy PA, Jessell TM: **Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of Sonic hedgehog proteolysis.** *Cell* 1995, **81**:445–455.
10. Hynes M, Porter JA, Chiang C, Chang D, Tessier-Lavigne M, Beachy PA, Rosenthal A: **Induction of midbrain dopaminergic neurons by sonic hedgehog.** *Neuron* 1995, **15**:35–44.
11. Ericson J, Muhr J, Placzek M, Lints T, Jessell TM, Edlund T. **Sonic hedgehog induces the differentiation of ventral forebrain neurons: a common signal for ventral patterning within the neural tube.** *Cell* 1995, **81**:747–756.
12. Macdonald R, Xu Q, Barth KA, Mikkola I, Holder N, Fjose A, Wilson S: **Regulatory gene expression boundaries demarcate sites of neuronal differentiation in the embryonic zebrafish forebrain.** *Neuron* 1994, **13**:1039–1053.
13. Hatta K, Puschel AW, Kimmel C: **Midline signalling in the primordium of the zebrafish anterior central nervous system.** *Proc Natl Acad Sci USA* 1994, **91**:2061–2065.
14. Ekker SC, Ungar AR, Greenstein P, von Kessler DP, Porter J, Moon RT, Beachy PA: **Patterning activities of the vertebrate Hedgehog proteins in the developing eye and brain.** *Curr Biol* 1995, **5**:944–955.
15. Macdonald R, Barth KA, Xu Q, Holder N, Mikkola I, Wilson S: **Midline signalling is required for *Pax* gene regulation and patterning of the eyes.** *Development* 1995, **121**:3267–3278.
16. Guthrie S, Muchamore I, Kuroiwa A, Marshall h, Krumlauf R, Lumsden A: **Neuroectodermal autonomy of *Hox-2.9* expression revealed by rhombomere transpositions.** *Nature* 1992, **356**:157–159.
17. Simon H, Lumsden A: **Rhombomere-specific origin of the contralateral vestibulo-acoustic efferent neurons and their migration across the embryonic midline.** *Neuron* 1993, **11**:209–220.
18. Simon H, Hornbruch A, Lumsden A: **Independent assignment of antero-posterior and dorso-ventral positional values in the developing chick hindbrain.** *Curr Biol* 1995, **5**:205–214.
19. Simeone A, Avantaggiato V, Moroni MC, Mavilio F, Arra C, Cotelli F, Nigro V, Acampora D: **Retinoic acid induces stage-specific antero-posterior transformation of rostral central nervous system.** *Mech Dev* 1995, **51**:83–98.
20. Moury J, Jacobson A: **Neural fold formation at newly created boundaries between neural plate and epidermis in the axolotl.** *Dev Biol* 1989, **133**:44–57.
21. Moury J, Jacobson A: **The origins of neural crest cells in the axolotl.** *Dev Biol* 1990, **141**:243–253.
22. Selleck M, Bronner-Fraser M: **Origins of the avian neural crest: the role of neural plate-epidermal interactions.** *Development* 1995, **121**:525–538.
23. Dickinson ME, Selleck M, McMahon AP, Bronner-Fraser M: **Dorsalisation of the neural tube by the non-neural ectoderm.** *Development* 1995, **121**:2099–2106.
24. Liem KF, Tremml G, Roelink H, Jessell TM: **Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm.** *Cell* 1995, **82**:969–979.
25. Basler K, Edlund T, Jessell TM, Yamada T: **Control of cell pattern in the neural tube: regulation of cell differentiation by dorsalin-1, a novel TGFβ family member.** *Cell* 1993, **73**:687–702.

Andrew Lumsden and Anthony Graham, Department of Developmental Neurobiology and Department of Experimental Pathology, UMDS, Guy's Hospital, London SE1 9RT, UK.